



Germline *POLE* mutation in a child with hypermutated medulloblastoma and features of constitutional mismatch repair deficiency

Holly Lindsay,^{1,2} Sarah Scollon,^{1,2} Jacquelyn Reuther,³ Horatiu Voicu,³ Surya P. Rednam,^{1,2} Frank Y. Lin,^{1,2} Kevin E. Fisher,³ Murali Chintagumpala,^{1,2} Adekunle M. Adesina,^{1,3} D. Will Parsons,^{1,2,3,4} Sharon E. Plon,^{1,2,3,4} and Angshumoy Roy^{1,3}

¹Department of Pediatrics, Division of Hematology-Oncology, Baylor College of Medicine, Houston, Texas 77030, USA; ²Texas Children's Cancer Center, Texas Children's Hospital, Houston, Texas 77030, USA; ³Department of Pathology and Immunology, ⁴Department of Molecular and Human Genetics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77030, USA

Abstract Ultra-hypermutation (>100 mutations/Mb) is rare in childhood cancer genomes and has been primarily reported in patients with constitutional mismatch repair deficiency (CMMRD) caused by biallelic germline mismatch repair (MMR) gene mutations. We report a 5-yr-old child with classic clinical features of CMMRD and an ultra-hypermutated medulloblastoma with retained MMR protein expression and absence of germline MMR mutations. Mutational signature analysis of tumor panel sequencing data revealed a canonical DNA polymerase-deficiency-associated signature, prompting further genetic testing that uncovered a germline *POLE* p.A456P missense variant, which has previously been reported as a recurrent somatic driver mutation in cancers. This represents the earliest known onset of malignancy in a patient with a germline mutation in the *POLE* proofreading polymerase. The clinical features in this child, virtually indistinguishable from those of CMMRD, suggest that polymerase-proofreading deficiency should be considered in the differential diagnosis of CMMRD patients with retained MMR function.

Corresponding author:
aroy@bcm.edu

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INTRODUCTION

DNA replication fidelity in humans is preserved via two distinct repair mechanisms: polymerase proofreading and the mismatch repair (MMR) system (Rayner et al. 2016). Proofreading of newly synthesized DNA strands during replication is performed by the 3'–5' exonuclease activity of the Pol δ and Pol ϵ replicative polymerase catalytic subunits, POLD1 and POLE, respectively (Ganai and Johansson 2016; Rayner et al. 2016). The MMR pathway is a postreplicative error correction mechanism for mismatches evading polymerase proofreading and is primarily effected by MLH1, MSH2, MSH6, and PMS2 (Germano et al. 2018).

Although a functional deficiency in either pathway leads to a "mutator" cellular phenotype (Loeb 2016), the association between defective replication repair and cancer is better recognized for the MMR pathway, which is defective in two inherited cancer predisposition syndromes. Monoallelic heterozygous loss-of-function germline mutations in MMR genes

cause Lynch syndrome, an autosomal dominant disorder (Lynch et al. 2015). In contrast, biallelic (compound heterozygous or homozygous) loss-of-function germline mutations in MMR genes result in constitutional MMR deficiency syndrome (CMMRD), which is inherited in an autosomal recessive pattern (Bakry et al. 2014; Wimmer et al. 2014).

Despite a shared pathogenesis, Lynch syndrome and CMMRD are distinct in inheritance pattern, epidemiology, and clinical features (Abedalthagafi 2018). CMMRD is characterized by a higher frequency of brain and hematological malignancies and a markedly earlier age of onset (median age at first tumor is <10 yr) of cancer (Tabori et al. 2017). In addition, there are nonmalignant dermatological manifestations including pigmented skin papules and café au lait macules reminiscent of neurofibromatosis type 1 as well as multiple pilomatricomas. These clinical features have been proposed as a scoring system for further diagnostic workup that includes tumor/normal MMR staining by immunohistochemistry and/or microsatellite instability (MSI) evaluation followed by genetic analysis (Bakry et al. 2014; Wimmer et al. 2014; Tabori et al. 2017). Tumors in CMMRD patients are invariably “hypermutated” (defined as >10 mutations/Mb) and often “ultra-hypermutated” (>100 mutations/Mb) with acquisition of secondary somatic *POLE/POLD1* mutations (Shlien et al. 2015; Bouffet et al. 2016), and characteristic mutational signatures of MMR deficiency may be detected in the tumor genome (Alexandrov et al. 2013).

Recently, a highly penetrant dominant cancer predisposition syndrome was described in kindreds with colonic polyposis and/or carcinomas in germline carriers of *POLE/POLD1* exonuclease domain mutations (EDMs) (Briggs and Tomlinson 2013; Palles et al. 2013). Initially termed polymerase proofreading-associated polyposis, emerging reports of extracolonic malignancies, including central nervous system (CNS) gliomas as well as pancreatic and endometrial adenocarcinomas (Rohlin et al. 2014; Hansen et al. 2015; Spier et al. 2015; Bellido et al. 2016) suggests that the *POLE/POLD1* cancer predisposition syndrome is an evolving entity. *POLE/POLD1* syndrome patients typically receive their first cancer diagnosis in adulthood (median 40 and 32.5 yr for *POLE* and *POLD1*, respectively), which is more similar to Lynch syndrome than CMMRD (Bellido et al. 2016). Tumors with *POLE/POLD1* EDMs (Cancer Genome Atlas Network 2012; Church et al. 2013) are typically MMR-proficient and microsatellite stable (MSS) and are ultra-hypermutated with a characteristic genomic mutational signature (Alexandrov et al. 2013; Rayner et al. 2016; Campbell et al. 2017). Recurrent somatic polymerase EDMs are more commonly reported in *POLE* than *POLD1*, with certain hotspot codons (D275, P286, S297, V411, P436, A456, Y458, S459, and S461) associated with very high mutation burdens (Campbell et al. 2017). Germline EDMs in familial cases, however, are highly restricted to only a handful of recurrent missense changes including *POLE* L242V and in certain *POLD1* codons (S478, L474, D316, and R409) (Bellido et al. 2016).

In this report, we present a 5-yr-old child with medulloblastoma and clinical features of CMMRD, whose ultra-hypermutated tumor was found to retain MMR expression and harbor a mutation signature of germline *POLE* deficiency. Genetic workup revealed an absence of germline MMR mutations and the discovery of a heterozygous germline *POLE* p.A456P mutation.

RESULTS

Clinical Presentation and Family History

The patient is a Latina female who presented at 5 yr of age with a several week progressive history of headache, nausea, vomiting, impaired balance, and frequent falling toward her right side. Initial physical examination revealed more than 100 hyperpigmented macules and papules over her entire body, including some with classic café au lait macule features (Fig. 1A,B). Maternal family history was negative for relatives with any early-onset cancers,

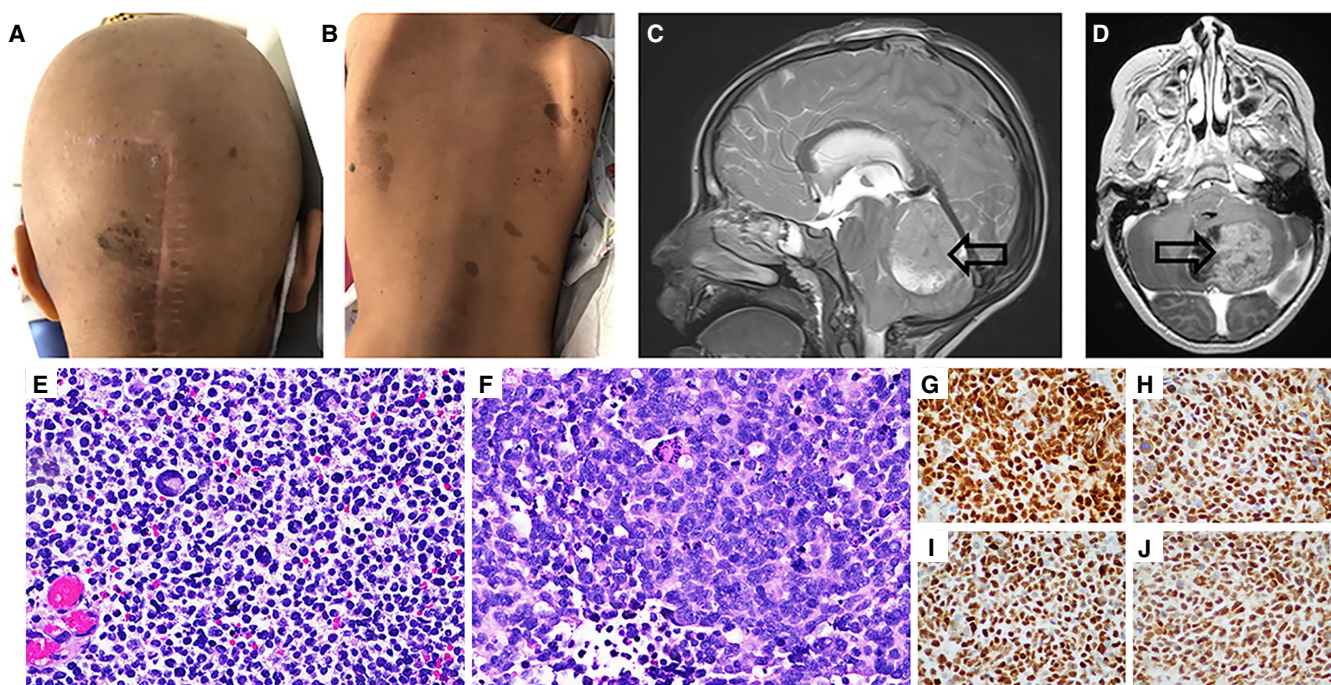


Figure 1. Representative images of numerous hyperpigmented skin lesions on the head (A) and back (B). Representative sagittal (C) and axial (D) MRI images showing a posterior fossa mass. Representative low-power (E) and high-power (F) images of the anaplastic medulloblastoma specimen demonstrating small round cells, mitotic figures, and scattered multinucleated giant cells. Immunohistochemistry for MMR proteins (G–J) revealing retained expression of MLH1, MSH2, MSH6, and PMS2, respectively.

and both maternal half-siblings are healthy without chronic medical conditions or hyperpigmented skin lesions. Paternal family and medical history were unavailable.

A head CT revealed a posterior fossa mass and obstructive hydrocephalus. Subsequent MRI confirmed the presence of a left cerebellar heterogeneously enhancing tumor with restricted diffusion, in addition to multifocal leptomeningeal metastatic disease along the cerebellum (Fig. 1C,D). The patient was additionally noted to have left-sided complex renal cyst, a right distal tibia osteochondroma, and three superficial hard palpable subcutaneous calcified nodules on her extremities, which were subsequently diagnosed as pilomatricomas on pathology examination.

A gross total resection of the primary posterior fossa tumor was performed. Pathologic examination revealed a large cell anaplastic medulloblastoma with several atypical mitotic figures, apoptosis, regional necrosis, and giant multinucleated cells (Fig. 1E,F). Additional medulloblastoma subclassification using fluorescence in situ hybridization probes for Chromosome 6q23 (monosomy 6), *MYC*, *MYCN*, *EGFR*, *PTEN* (monosomy 10), and immunohistochemistry (GLI1, YAP1, β -catenin, p53, FOXG1) revealed a non-WNT/non-SHH medulloblastoma without *MYC/MYCN* amplification. MMR immunohistochemistry of the tumor revealed staining for all four MMR proteins to be strongly retained in both tumor and normal cells (Fig. 1G–J).

Genomic Analyses

Given the suspected diagnosis of CMMRD, genetic analysis was performed on both germline and tumor specimens. Germline analysis of MMR pathway genes (*MLH1*, *MSH2*, *MSH6*,

Table 1. Panel genes tested for pathogenic germline variants in the patient

Panel	Genes
Hereditary high-risk CRC panel	<i>APC, BMPR1A, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53</i>
Hereditary brain/CNS/PNS cancer panel (unique genes not on CRC panel)	<i>ALK, ATM, MEN1, MRE11A, NBN, NF2, PALB2, PHOX2B, PTCH1, SUFU, VHL</i>

(CRC) Colorectal cancer, (CNS) central nervous system, (PNS) peripheral nervous system.

and *PMS2*) and other genes associated with childhood brain tumors (*TP53, PTCH1, NF1, APC*, etc.) (Table 1) using gene panels featuring variant and deletion/duplication analysis (including for *EPCAM*) was negative for any pathogenic or likely pathogenic variants or copy-number abnormalities.

Tumor variant analysis was undertaken on the medulloblastoma specimen using a 124-gene next-generation sequencing (NGS) panel in a CLIA-certified laboratory (Supplemental Table S1A) revealing several loss-of-function variants in genes commonly mutated in medulloblastoma (Table 2), including pathogenic nonsense mutations in *TP53* and *APC* that, as noted above, were absent in the germline of the patient in testing for familial cancer predisposition (Table 1), confirming a somatic origin for these mutations. The key finding, however, was that of an ultra-hypermutated tumor with a markedly increased tumor mutation burden (TMB) estimated at >150 mutations/Mb of the genome. Notably, 72/94 variants on the tumor panel report had no entries in reference population databases with at least 25% (18/72) being nonsense mutations.

Given the profile of an MMR-proficient ultra-hypermutated tumor, the possibility of a polymerase proofreading error-related mechanism was entertained. Mutation signature analysis of all tumor point mutations and the surrounding trinucleotide context revealed a disproportionate frequency of C>A transversions (~35%) and C>T transitions (~50%), with the T[C>T]G (24%) and T[C>A]T (18%) signature together comprising >40% of the mutational burden (Fig. 2A). This mutational pattern is characteristic of COSMIC “signature 10” (Alexandrov et al. 2013; Rayner et al. 2016) and an unsupervised similarity analysis of the tumor mutational profile to all published signature patterns confirmed the highest cosine similarity of 0.87 (1 being identical) to that signature (Fig. 2B).

Because of the known association between signature 10 and germline *POLE* deficiency, a 981-gene comprehensive cancer panel (Supplemental Table S1B) containing *POLE/POLD1* and MMR genes was used to sequence genomic DNA from germline and tumor specimens. The analysis revealed a heterozygous germline missense mutation in exon 14 of *POLE*: NM_006231.3(*POLE*):c.1366G>C (p.Ala456Pro) (Table 3; Fig. 2C). This variant, previously reported as a recurrent somatic *POLE* driver mutation and associated with a

Table 2. Relevant tumor panel findings

Gene	hg19 position	CDS change	AA change	Variant type	Origin	dbSNP	Variant allele fraction	COSMIC ID
<i>APC</i>	Chr 5:112175124	c.3833C>A	p.Ser1278Ter	Nonsense	Somatic		27.40%	COSM19313
<i>KDM6A</i>	Chr X:44950066	c.3835C>T	p.Arg1279Ter	Nonsense	Somatic	rs863224886	50.00%	COSM28780
<i>NF2</i>	Chr 22:30070832	c.1348G>T	p.Glu450Ter	Nonsense	Somatic		49.60%	
<i>PTCH1</i>	Chr 9:98244436	c.634G>T	p.Glu212Ter	Nonsense	Somatic		43.70%	
<i>SUFU</i>	Chr 10:104387001	c.1365+1G>T		Splicing	Somatic		46.30%	
<i>TP53</i>	Chr 17:7578212	c.637C>T	p.Arg213Ter	Nonsense	Somatic	rs397516436	98.80%	COSM10654

(CDS) Coding sequence, (AA) amino acid.

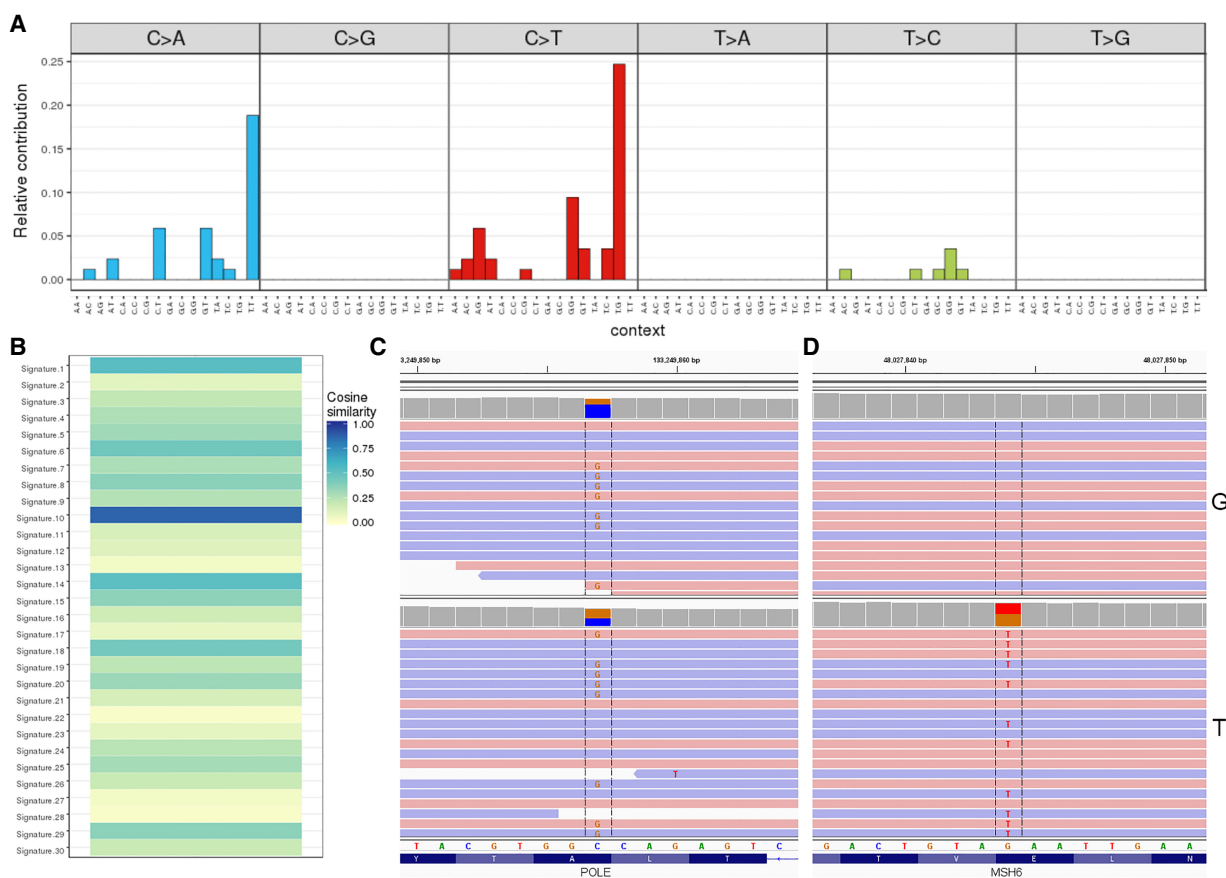


Figure 2. Mutation signature analysis and identification of germline *POLE* mutation. Mutation signature profile of the tumor as represented on the 96-substitution classification (A) along with the trinucleotide context. Mutations and the contexts are plotted on the horizontal axis, and the vertical axis represents the proportional contribution of each pattern to the overall signature. A striking pattern of increased C>A [TCT] and C>T [TCG] trinucleotide contexts forming ~40% of all changes is observed, which has been strongly associated with “signature 10,” and confirmed on mutational pattern similarity analysis against all 30 COSMIC signatures (B) showing closest match to “signature 10” (cosine similarity 0.87). The signature prediction of germline *POLE* alteration was corroborated by germline and tumor sequencing revealing a *POLE* p.A456P mutation (C) in the germline (top panel, “G”) and tumor (bottom panel, “T”). In contrast, a heterozygous *MSH6* nonsense mutation was only seen in the tumor (D).

high mutation burden (Campbell et al. 2017), lies within the Exo III motif of the *POLE* exonuclease domain in close proximity to several recurrent somatic *POLE* mutations. The p.A456P mutation alters a highly conserved alanine residue that is predicted to affect DNA binding and lead to defective proofreading activity (Church et al. 2013). No additional somatic *POLE* mutation (or copy-number change and/or LOH) as a second hit was noted. Further genetic testing for *POLE* in the mother revealed that she did not harbor the germline p.A456P variant. Repeated attempts by the clinical team to reach the father for familial testing were unsuccessful; thus, a paternally inherited mutation cannot be ruled out.

The comprehensive cancer panel testing of the tumor also revealed a nonsense mutation in exon 4 of *MSH6* that was not present in the germline: NM_000179.2(*MSH6*):c.2722G>T (p.Glu908Ter) at 46.7% variant allele fraction (Fig. 2D). No second hit in *MSH6* or other alterations in the MMR genes were detected, consistent with the retention of MMR staining in the cells (Fig. 1G–J). Both the germline *POLE* and somatic *MSH6* variants were independently

Table 3. Germline *POLE* and somatic *MSH6* variants detected in the patient

Gene	hg19 position	Depth at position (G, T)	CDS change	AA change	Variant type	Origin	Predicted effect	dbSNP	Geno	ClinVar ID
<i>POLE</i>	12:133249857	51, 136	c.1366G>C	p.A456P	Missense	G	Del	–	Het	SCV000825544.1
<i>MSH6</i>	2:48027844	302, 1004	c.2722G>T	p.Q908X	Nonsense	S	Del	–	Het	

(CDS) Coding sequence, (AA) amino acid, (G) germline, (S) somatic, (T) tumor, (Del) deleterious, (Het) heterozygous.

confirmed by direct Sanger sequencing. No other pathogenic alterations in any of the remaining polymerase genes were detected.

Clinical Course

Following clinical recovery from gross total resection of the primary tumor, the patient was treated with proton beam craniospinal irradiation (at 3600 cobalt Gray equivalent plus boost to tumor bed and nodules) followed by seven cycles of chemotherapy (including vincristine, cisplatin, cyclophosphamide, gemcitabine, and pemetrexed) on a high-risk arm of a multi-institutional phase 2 clinical trial for patients with newly diagnosed medulloblastoma. Her chemotherapy courses were complicated by malnutrition necessitating enteral caloric supplementation via nasogastric tube and recurrent episodes of *Clostridium difficile* colitis and methicillin-susceptible *Staphylococcus aureus* bacteremia. She otherwise tolerated her treatment without severe complication and is doing well without evidence of medulloblastoma recurrence or progression of leptomeningeal metastasis 4 mo after completion of treatment.

Clinical experts in pediatric cancer surveillance were consulted to devise a customized screening protocol including at least annual brain MRI imaging indefinitely given the need for surveillance for recurrence as well as new *POLE* polymerase cancer syndrome–related brain tumors. She additionally will soon be undergoing a baseline colonoscopy. The timing of subsequent serial evaluations will be based on the presence or absence of colon adenomas; if no adenomas are identified, a repeat colonoscopy can be delayed until the patient becomes a teenager. She is followed biannually by dermatology and will undergo annual gynecology exams on reaching adulthood.

DISCUSSION

This case illustrates several novel and striking aspects of the still-evolving *POLE/POLD1* polymerase cancer syndrome as well as the utility of integrative tumor sequencing and mutation signature analysis in accurately predicting the underlying mutational mechanism in a tumor.

First, the patient presented with clinical features indistinguishable from that of CMMRD based on current diagnostic criteria proposed by the C4CMMRD consortium (Wimmer et al. 2014), including a pediatric-onset tumor (1 point), numerous café au lait macules (2 points), and multiple pilomatricomas (2 points), with a score of ≥ 3 suggested to prompt screening for MMR deficiency (MMR-D). Other investigators have recently reported a 14-yr-old boy with rectosigmoid carcinoma and CMMRD-like dermatological manifestations and a germline *POLE* missense mutation (p.V411L) (Wimmer et al. 2017), suggesting that the characteristic skin manifestations of CMMRD may be common to different DNA repair defect syndromes. Our results corroborate that and provide further evidence that a *POLE/POLD1* deficiency should be considered in the differential of CMMRD patients, particularly if further testing excludes MMR deficiency.

Second, to our knowledge, at age 5 yr, this is the youngest cancer presentation reported in a patient with germline *POLE/POLD1* cancer predisposition syndrome. Except for the 14-yr-old boy described by Wimmer et al. (2017) with a negative family history, to date, the median age at first cancer diagnosis in all known familial cases of *POLE/POLD1* syndrome is reported to be in the fourth decade (earliest 27 yr) (Bellido et al. 2016). The presentation in early childhood is, therefore, highly unusual and contributed to further overlap with the clinical presentation of CMMRD.

Third, this is the first report of the *POLE* p.A456P mutation as a germline alteration. The p.A456P EDM is a known recurrent somatic hotspot mutation that has been previously reported in at least 32 tumors (Table 4) (COSM937319), including hyper/ultra-hypermutated tumors with mutational signatures characteristic of *POLE* proofreading deficiency (Shinbrot et al. 2014). Review of the literature reveals that the TMB, when reported, is invariably high in all tumors harboring the p.A456P mutation (Table 4). The alanine at codon 456 is highly conserved from humans to *Drosophila*, and structural analysis predicts mutation of this site to lead to altered DNA binding through distortion of the polymerase active site (Church et al. 2013). Taken together, the p.A456P EDM is considered a driver *POLE* mutation in human carcinogenesis (Campbell et al. 2017) and is the most likely cause of the phenotype in this patient. As important, the mutation was found to be absent in the germline of the asymptomatic mother, although paternal inheritance could not be ruled out.

The combination of an unusually early age of onset with a novel germline occurrence of a recurrent somatic mutation raises the possibility of the p.A456P EDM leading to a more severe *POLE* proofreading defect. It is noteworthy that in a large screen of cancers for driver *POLE* EDMs (Campbell et al. 2017), both the p.A456P mutation and the p.V411L mutation reported previously in the 14-yr-old boy (Wimmer et al. 2017) were characterized as strong “mutators” because of a tight correlation with elevated TMB. In contrast, using functional assays and association with TMB, the only recurrent familial germline *POLE* mutation (p.L424V) was found to have a weak “mutator” phenotype (Shinbrot et al. 2014; Campbell et al. 2017), possibly correlating with a later age of onset seen in those individuals. Further reports of other *POLE* EDMs in the germline along with functional studies would be necessary to clarify the genotype–phenotype correlation between the severity of the *POLE* mutation and age of onset.

Ultra-hypermutation was first recognized in adult-onset MSS colon cancers that were found to be enriched for *POLE* and, less commonly, *POLD1* EDMs (Cancer Genome Atlas Network 2012; Donehower et al. 2013; Palles et al. 2013). Childhood cancer genomes are typically characterized by a low TMB (Vogelstein et al. 2013; Schlesner and Eils 2015) with

Table 4. Reported *POLE* p.A456P tumor mutations and associated mutation burden

Tumor	Total cases	TMB-ultra	TMB-hyper	TMB-low	TMB-ND	Reference(s)
Colorectal carcinoma	7	5	1	-	1	Shinbrot et al. 2014; Stenzinger et al. 2014; Jansen et al. 2016; Campbell et al. 2017
Endometrial carcinoma	21	2	2	-	17	Church et al. 2013; Kandoth et al. 2013; Billingsley et al. 2015; Talhouk et al. 2015; Espinosa et al. 2016; McConechy et al. 2016; Bellone et al. 2017; Campbell et al. 2017; Espinosa et al. 2017; Van Gool et al. 2018
Glioblastoma multiforme	1	1	-	-	-	Hoadley et al. 2018
Ovarian carcinoma	2	-	1	-	1	Campbell et al. 2017; Parra-Herran et al. 2017
Cervical squamous cell carcinoma	1	-	1	-	-	Campbell et al. 2017

(TMB) Tumor mutation burden, (hyper) hypermutated (>10/Mb), (ultra) ultra-hypermutated (>100/Mb), (ND) not determined.

hyper/ultra-hypermutation seen in <1% of all pediatric cancers, primarily in the context of CMMRD (Gröbner et al. 2018). Tumor profiling using NGS panels to direct management of cancers is now common in clinical practice, and this report highlights the utility of tumor sequencing and analysis. Detection of an ultra-hypermutated tumor has potentially major therapeutic implications for this patient as a positive association between TMB and the response to therapeutic immune checkpoint inhibition, including improved progression-free survival and increased rates of objective response, presumed secondary to an increased number of neoantigens in hypermutated tumors (Rizvi et al. 2015; Bourdais et al. 2017; Le et al. 2017; Nebot-Bral et al. 2017), is now well-established. In the event of tumor recurrence in this patient, treatment with a PD-1 or PD-L1 inhibitor will be strongly considered. Multiple clinical trials of immune checkpoint inhibitors specifically enrolling patients with hypermutated tumors are currently available (Clinical Trials NCT02359565, NCT02992964).

In addition, the identification of ultra-hypermutation on tumor panel analysis in the absence of MMR-D (as evidenced by negative MMR genetic testing and retained MMR expression by IHC) for the first time raised the possibility of a germline *POLE* deficiency as the primary genetic defect, despite the unusually young age at presentation for that diagnosis. The tumor mutational signature analysis revealed an unambiguous “signature 10” pattern that is a “canonical” signature of *POLE* deficiency (Alexandrov et al. 2013; Campbell et al. 2017). This directed further evaluation of the tumor and germline specimens for *POLE/POLD1* mutations revealing the deleterious *POLE* p.A456P mutation in the germline. This report corroborates the accuracy of mutational signature analysis of hypermutated tumors from tumor panel sequencing data in prospectively predicting the underlying genetic defect, as proposed recently in the form of a diagnostic algorithm for hypermutated childhood tumors (Campbell et al. 2017). The correlations reported here and elsewhere (Campbell et al. 2017) emphasize that germline testing for CMMRD and *POLE/POLD1* inherited cancer syndrome may result from either clinical features or tumor mutation spectrum (when available). Notably, identification of the germline *POLE* defect in our patient along with the reported pediatric CRC case (Wimmer et al. 2017) has also led to modification in the follow-up care, as current cancer screening recommendations for patients with germline *POLE* mutations include colonoscopies every 1–2 yr and gastroduodenoscopies every 3 yr beginning at 18–25 yr old (Spier et al. 2015; Bellido et al. 2016; Rayner et al. 2016; Buchanan et al. 2018).

Finally, although both colonic and extracolonic malignancies, including CNS astrocytomas, have been previously reported in familial *POLE/POLD1* mutation carriers, (Rohlin et al. 2014; Hansen et al. 2015; Spier et al. 2015; Bellido et al. 2016; Johanns et al. 2016; Esteban-Jurado et al. 2017), to our knowledge, this is the first report of a medulloblastoma as the presenting malignancy in a patient with germline *POLE* mutation. Except for the scattered giant multinucleated cells that have been previously reported in hypermutated glioblastomas with somatic *POLE* mutations (Erson-Omay et al. 2015), the histopathological characteristics of the anaplastic medulloblastoma were characteristic. Our report, therefore, extends both the age of diagnosis and the spectrum of tumors seen in *POLE/POLD1* syndrome patients and suggests that the phenotype of this newly described cancer predisposition syndrome is still being defined.

METHODS

The study was performed after obtaining informed consent for enrollment on a Baylor College of Medicine Institutional Review Board-approved study.

Genomic DNA was extracted from blood and the medulloblastoma specimen utilizing the QIAamp DNA Mini Kit and the DNA FFPE Tissue Kit (QIAGEN), respectively.

Table 5. Sequencing metrics on the human oncology panel for the tumor and germline specimens

Sample	Total reads	Percent aligned reads	Percent reads on target	Average coverage
Germline	7,096,416	94.29%	68.95%	181.92
Tumor	20,774,903	95.49%	77.56%	581.33

Germline DNA underwent clinical sequencing at two CLIA-certified reference laboratories: Baylor Genetics laboratory for the Hereditary High-risk CRC panel and the Hereditary Brain/CNS/PNS panel and at Invitae laboratories for *POLE* testing. Additional sequencing using the SeqCap EZ Human Oncology Panel (Roche Inc.) was performed at Texas Children's Hospital. Tumor DNA underwent clinical sequencing at the CLIA-certified Texas Children's Hospital laboratory using the Solid Tumor NGS panel as well as the Human Oncology Panel. A list of genes covered on the tumor panels is included in Supplemental Table S1.

Sequencing by the hereditary cancer panels at Baylor Genetics featured Illumina-based NGS variant analysis and MLPA/CGH-based deletion/duplication analysis of familial cancer genes (Table 1). For the human oncology panel and solid tumor panels, NGS libraries were generated using 50 ng of DNA and subsequently pooled in either 4-plex or 2-plex for hybridization capture with respective capture probe set. Captured pools were then sequenced on an Illumina MiSeq with 600V3 chemistry (2 × 150-bp read length). Coverage metrics for the sequenced samples are provided in Table 5. NextGENe (SoftGenetics) and Platypus (Rimmer et al. 2014) were utilized for variant calling, followed by annotation with VEP (McLaren et al. 2016). Mutation prediction algorithms (PolyPhen, SIFT, LRT, MutationTaster) were used using dbNSFP (Liu et al. 2011) to predict the biological effect of the mutation.

Mutation signature analysis and visualization were generated using the Mutational Patterns Bioconductor package in R (Blokzijl et al. 2018). The package was utilized to generate the 96-substitution classification profile (Alexandrov et al. 2013) with all substitutions converted to the pyrimidine of the Watson–Crick base pair and representing the immediate trinucleotide context (5' × 3') of the mutated base. The optimal nonnegative linear combination of COSMIC signatures was then fitted to the substitution classification profile, followed by calculation of the cosine similarity between the tumor mutation signature in this patient and the COSMIC signatures to determine the mutation signature classes that best fit the signature of the patient.

Tumor mutation burden was calculated from the solid tumor mutation panel (~1 Mb capture size) using the total number of nonsynonymous variants in the coding region of the panel adjusted for the panel size.

ADDITIONAL INFORMATION

Data Deposition and Access

The *POLE* variant can be found in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) under accession number SCV000825544.1. The *MSH6* variant was submitted to ClinVar and can be found under accession number SCV000965671.1. Tumor and germline targeted sequencing data from the human oncology panel will be deposited to the National Center for Biotechnology Information (NCBI) Database of Genotypes and Phenotypes (dbGaP; <http://www.ncbi.nlm.nih.gov/gap>).

Ethics Statement

Informed consent for enrollment on study H-27233 “Molecular Characterization of Cancer Genomes” approved by the Institutional Review Board of Baylor College of Medicine was obtained by a study physician.

Author Contributions

Clinical care was provided by H.L., S.S., S.P.R., M.C., A.M.A., and S.E.P. Genomic data analysis and interpretation were performed by S.S., J.R., H.V., F.Y.L., K.E.F., D.W.P., S.E.P., and A.R. H.L. and A.R. wrote the initial draft of the manuscript. All authors contributed to reviewing and editing the final draft.

Competing Interest Statement

The authors have declared no competing interest.

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REFERENCES

- Abedalthagafi M. 2018. Constitutional mismatch repair-deficiency: current problems and emerging therapeutic strategies. *Oncotarget* **9**: 35458–35469. doi:10.18632/oncotarget.26249
- Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale AL, et al. 2013. Signatures of mutational processes in human cancer. *Nature* **500**: 415–421. doi:10.1038/nature12477
- Bakry D, Aronson M, Durno C, Rimawi H, Farah R, Alharbi QK, Alharbi M, Shamvil A, Ben-Shachar S, Mistry M, et al. 2014. Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. *Eur J Cancer* **50**: 987–996. doi:10.1016/j.ejca.2013.12.005
- Bellido F, Pineda M, Aiza G, Valdés-Mas R, Navarro M, Puente DA, Pons T, González S, Iglesias S, Darder E, et al. 2016. *POLE* and *POLD1* mutations in 529 kindred with familial colorectal cancer and/or polyposis: review of reported cases and recommendations for genetic testing and surveillance. *Genet Med* **18**: 325–332. doi:10.1038/gim.2015.75
- Bellone S, Bignotti E, Lonardi S, Ferrari F, Centritto F, Masserdotti A, Pettinella F, Black J, Menderes G, Altwerger G, et al. 2017. Polymerase ϵ (*POLE*) ultra-mutation in uterine tumors correlates with T lymphocyte infiltration and increased resistance to platinum-based chemotherapy in vitro. *Gynecol Oncol* **144**: 146–152. doi:10.1016/j.ygyno.2016.11.023
- Billingsley CC, Cohn DE, Mutch DG, Stephens JA, Suarez AA, Goodfellow PJ. 2015. Polymerase varepsilon (*POLE*) mutations in endometrial cancer: clinical outcomes and implications for Lynch syndrome testing. *Cancer* **121**: 386–394. doi:10.1002/cncr.29046
- Blokzijl F, Janssen R, van Boxtel R, Cuppen E. 2018. MutationalPatterns: comprehensive genome-wide analysis of mutational processes. *Genome Med* **10**: 33. doi:10.1186/s13073-018-0539-0
- Bouffet E, Larouche V, Campbell BB, Merico D, de Borja R, Aronson M, Durno C, Krueger J, Cabric V, Ramaswamy V, et al. 2016. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. *J Clin Oncol* **34**: 2206–2211. doi:10.1200/JCO.2016.66.6552
- Bourdais R, Rousseau B, Pujals A, Boussion H, Joly C, Guillemin A, Baumgaertner I, Neuzillet C, Tournigand C. 2017. Polymerase proofreading domain mutations: new opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. *Crit Rev Oncol Hematol* **113**: 242–248. doi:10.1016/j.critrevonc.2017.03.027
- Briggs S, Tomlinson I. 2013. Germline and somatic polymerase ϵ and δ mutations define a new class of hypermutated colorectal and endometrial cancers. *J Pathol* **230**: 148–153. doi:10.1002/path.4185
- Buchanan DD, Stewart JR, Clendenning M, Rosty C, Mahmood K, Pope BJ, Jenkins MA, Hopper JL, Southey MC, Macrae FA, et al. 2018. Risk of colorectal cancer for carriers of a germ-line mutation in *POLE* or *POLD1*. *Genet Med* **20**: 890–895. doi:10.1038/gim.2017.185
- Campbell BB, Light N, Fabrizio D, Zatzman M, Fuligni F, de Borja R, Davidson S, Edwards M, Elvin JA, Hodel KP, et al. 2017. Comprehensive analysis of hypermutation in human cancer. *Cell* **171**: 1042–1056.e10. doi:10.1016/j.cell.2017.09.048

- Cancer Genome Atlas Network. 2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**: 330–337. doi:10.1038/nature11252
- Church DN, Briggs SEW, Palles C, Domingo E, Kearsley SJ, Grimes JM, Gorman M, Martin L, Howarth KM, Hodgson SV, et al. 2013. DNA polymerase ϵ and δ exonuclease domain mutations in endometrial cancer. *Hum Mol Genet* **22**: 2820–2828. doi:10.1093/hmg/ddt131
- Donehower LA, Creighton CJ, Schultz N, Shinbrot E, Chang K, Gunaratne PH, Muzny D, Sander C, Hamilton SR, Gibbs RA, et al. 2013. *MLH1*-silenced and non-silenced subgroups of hypermutated colorectal carcinomas have distinct mutational landscapes. *J Pathol* **229**: 99–110. doi:10.1002/path.4087
- Erson-Omay EZ, Çağlayan AO, Schultz N, Weinhold N, Omay SB, Özdoğan K, Köksal Y, Li J, Serin Harmanci A, Clark V, et al. 2015. Somatic *POLE* mutations cause an ultramutated giant cell high-grade glioma subtype with better prognosis. *Neuro Oncol* **17**: 1356–1364. doi:10.1093/neuonc/nov027
- Espinosa I, D'Angelo E, Palacios J, Prat J. 2016. Mixed and ambiguous endometrial carcinomas: a heterogeneous group of tumors with different clinicopathologic and molecular genetic features. *Am J Surg Pathol* **40**: 972–981. doi:10.1097/PAS.0000000000000640
- Espinosa I, Lee CH, D'Angelo E, Palacios J, Prat J. 2017. Undifferentiated and dedifferentiated endometrial carcinomas with *POLE* exonuclease domain mutations have a favorable prognosis. *Am J Surg Pathol* **41**: 1121–1128. doi:10.1097/PAS.0000000000000873
- Esteban-Jurado C, Giménez-Zaragoza D, Muñoz J, Franch-Expósito S, Álvarez-Barona M, Ocaña T, Cuatrecasas M, Carballal S, López-Ceron M, Marti-Solano M, et al. 2017. *POLE* and *POLD1* screening in 155 patients with multiple polyps and early-onset colorectal cancer. *Oncotarget* **8**: 26732–26743. doi:10.18632/oncotarget.15810
- Ganai RA, Johansson E. 2016. DNA replication—a matter of fidelity. *Mol Cell* **62**: 745–755. doi:10.1016/j.molcel.2016.05.003
- Germano G, Amirouchene-Angelozzi N, Rospo G, Bardelli A. 2018. The clinical impact of the genomic landscape of mismatch repair-deficient cancers. *Cancer Discov* **8**: 1518–1528. doi:10.1158/2159-8290.CD-18-0150
- Gröbner SN, Worst BC, Weischenfeldt J, Buchhalter I, Kleinheinz K, Rudneva VA, Johann PD, Balasubramanian GP, Segura-Wang M, Brabetz S, et al. 2018. The landscape of genomic alterations across childhood cancers. *Nature* **555**: 321–327. doi:10.1038/nature25480
- Hansen MF, Johansen J, Bjørnevoll I, Sylvander AE, Steinsbekk KS, Sætrum P, Sandvik AK, Drabløs F, Sjørusen W. 2015. A novel *POLE* mutation associated with cancers of colon, pancreas, ovaries and small intestine. *Fam Cancer* **14**: 437–448. doi:10.1007/s10689-015-9803-2
- Hoadley KA, Yau C, Hinoue T, Wolf DM, Lazar AJ, Drill E, Shen R, Taylor AM, Cherniack AD, Thorsson V, et al. 2018. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell* **173**: 291–304.e6. doi:10.1016/j.cell.2018.03.022
- Jansen AML, van Wezel T, van den Akker BEWM, Ventayol Garcia M, Ruano D, Tops CMJ, Wagner A, Letteboer TGW, Gómez-García EB, Devilee P, et al. 2016. Combined mismatch repair and *POLE/POLD1* defects explain unresolved suspected Lynch syndrome cancers. *Eur J Hum Genet* **24**: 1089–1092. doi:10.1038/ejhg.2015.252
- Johanns TM, Miller CA, Dorward IG, Tsien C, Chang E, Perry A, Uppaluri R, Ferguson C, Schmidt RE, Dahiya S, et al. 2016. Immunogenomics of hypermutated glioblastoma: a patient with germline *POLE* deficiency treated with checkpoint blockade immunotherapy. *Cancer Discov* **6**: 1230–1236. doi:10.1158/2159-8290.CD-16-0575
- Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, et al. 2013. Mutational landscape and significance across 12 major cancer types. *Nature* **502**: 333–339. doi:10.1038/nature12634
- Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, et al. 2017. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* **357**: 409–413. doi:10.1126/science.aan6733
- Liu X, Jian X, Boerwinkle E. 2011. dbNSFP: a lightweight database of human nonsynonymous SNPs and their functional predictions. *Hum Mutat* **32**: 894–899. doi:10.1002/humu.21517
- Loeb LA. 2016. Human cancers express a mutator phenotype: hypothesis, origin, and consequences. *Cancer Res* **76**: 2057–2059. doi:10.1158/0008-5472.CAN-16-0794
- Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. 2015. Milestones of lynch syndrome: 1895–2015. *Nat Rev Cancer* **15**: 181–194. doi:10.1038/nrc3878
- McConechy MK, Talhouk A, Leung S, Chiu D, Yang W, Senz J, Reha-Krantz LJ, Lee C-H, Huntsman DG, Gilks CB, et al. 2016. Endometrial carcinomas with *POLE* exonuclease domain mutations have a favorable prognosis. *Clin Cancer Res* **22**: 2865–2873. doi:10.1158/1078-0432.CCR-15-2233
- McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GRS, Thormann A, Flicek P, Cunningham F. 2016. The Ensembl Variant Effect Predictor. *Genome Biol* **17**: 122. doi:10.1186/s13059-016-0974-4

- Nebot-Bral L, Brandao D, Verlingue L, Rouleau E, Caron O, Despras E, El-Dakdouki Y, Champiat S, Aoufouchi S, Leary A, et al. 2017. Hypermutated tumours in the era of immunotherapy: the paradigm of personalised medicine. *Eur J Cancer* **84**: 290–303. doi:10.1016/j.ejca.2017.07.026
- Palles C, Cazier JB, Howarth KM, Domingo E, Jones AM, Broderick P, Kemp Z, Spain SL, Guarino E, Salguero I, et al. 2013. Germline mutations affecting the proofreading domains of *POLE* and *POLD1* predispose to colorectal adenomas and carcinomas. *Nat Genet* **45**: 136–144. doi:10.1038/ng.2503
- Parra-Herran C, Lerner-Ellis J, Xu B, Khalouei S, Bassiouny D, Cesari M, Ismiil N, Nofech-Mozes S. 2017. Molecular-based classification algorithm for endometrial carcinoma categorizes ovarian endometrioid carcinoma into prognostically significant groups. *Mod Pathol* **30**: 1748–1759. doi:10.1038/modpathol.2017.81
- Rayner E, van Gool IC, Palles C, Kearsley SE, Bosse T, Tomlinson I, Church DN. 2016. A panoply of errors: polymerase proofreading domain mutations in cancer. *Nat Rev Cancer* **16**: 71–81. doi:10.1038/nrc.2015.12
- Rimmer A, Phan H, Mathieson I, Iqbal Z, Twigg SRF, Consortium WGS, Wilkie AOM, McVean G, Lunter G. 2014. Integrating mapping-, assembly- and haplotype-based approaches for calling variants in clinical sequencing applications. *Nat Genet* **46**: 912–918. doi:10.1038/ng.3036
- Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, et al. 2015. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**: 124–128. doi:10.1126/science.aaa1348
- Rohlin A, Zagoras T, Nilsson S, Lundstam ULF, Wahlström J, Hultén L, Martinsson T, Karlsson GB, Nordling M. 2014. A mutation in *POLE* predisposing to a multi-tumour phenotype. *Int J Oncol* **45**: 771.1. doi:10.3892/ijo.2014.2410
- Schlesner M, Eils R. 2015. Hypermutation takes the driver's seat. *Genome Med* **7**: 31. doi:10.1186/s13073-015-0159-x
- Shinbrot E, Henninger EE, Weinhold N, Covington KR, Göksenin AY, Schultz N, Chao H, Doddapaneni H, Muzny DM, Gibbs RA, et al. 2014. Exonuclease mutations in DNA polymerase ϵ reveal replication strand specific mutation patterns and human origins of replication. *Genome Res* **24**: 1740–1750. doi:10.1101/gr.174789.114
- Shlien A, Campbell BB, de Borja R, Alexandrov LB, Merico D, Wedge D, Van Loo P, Tarpey PS, Coupland P, Behjati S, et al. 2015. Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. *Nat Genet* **47**: 257–262. doi:10.1038/ng.3202
- Spier I, Holzapfel S, Altmüller J, Zhao B, Horpaopan S, Vogt S, Chen S, Morak M, Raeder S, Kayser K, et al. 2015. Frequency and phenotypic spectrum of germline mutations in *POLE* and seven other polymerase genes in 266 patients with colorectal adenomas and carcinomas. *Int J Cancer* **137**: 320–331. doi:10.1002/ijc.29396
- Stenzinger A, Pfarr N, Endris V, Penzel R, Jansen L, Wolf T, Herpel E, Warth A, Klauschen F, Kloor M, et al. 2014. Mutations in *POLE* and survival of colorectal cancer patients—link to disease stage and treatment. *Cancer Med* **3**: 1527–1538. doi:10.1002/cam4.305
- Tabori U, Hansford JR, Achatz MI, Kratz CP, Plon SE, Frebourg T, Brugières L. 2017. Clinical management and tumor surveillance recommendations of inherited mismatch repair deficiency in childhood. *Clin Cancer Res* **23**: e32–e37. doi:10.1158/1078-0432.CCR-17-0574
- Talhok A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, Yang W, Senz J, Boyd N, Karnezis AN, et al. 2015. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer* **113**: 299–310. doi:10.1038/bjc.2015.190
- Van Gool IC, Ubachs JEH, Stelloo E, de Kroon CD, Goeman JJ, Smit VTHBM, Creutzberg CL, Bosse T. 2018. Blinded histopathological characterisation of *POLE* exonuclease domain-mutant endometrial cancers: sheep in wolf's clothing. *Histopathology* **72**: 248–258. doi:10.1111/his.13338
- Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr., Kinzler KW. 2013. Cancer genome landscapes. *Science* **339**: 1546–1558. doi:10.1126/science.1235122
- Wimmer K, Kratz CP, Vasen HFA, Caron O, Colas C, Entz-Werle N, Gerdes AM, Goldberg Y, Ilencikova D, Muleris M, et al. 2014. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). *J Med Genet* **51**: 355–365. doi:10.1136/jmedgenet-2014-102284
- Wimmer K, Beilken A, Nustede R, Ripperger T, Lamottke B, Ure B, Steinmann D, Reineke-Plaass T, Lehmann U, Zschocke J, et al. 2017. A novel germline *POLE* mutation causes an early onset cancer prone syndrome mimicking constitutional mismatch repair deficiency. *Fam Cancer* **16**: 67–71. doi:10.1007/s10689-016-9925-1